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Surface Chemistry of Protein Adhesion Domains on Diblock Copolymer Films Characterized by Chemical Force Spectroscopy Mapping Technique

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Adhesions play an important role in adherent cell structures encouraging a variety of chemical and mechanical signals which, in turn, regulate cell behaviors including differentiation. In Vitro, cell substrates were modified by coating with protein ligands homogeneously, in cellular scale, allowing cell attachment and proliferation. However, extracellular matrices in reality provide heterogeneous adhesive sites. To mimic this, diblock copolymer films (dBCP) of polystyrene-block-polyacrylic acid (PS-b-PAA)and polystyrene-block-polyethylene oxide (PS-b-PEO) were introduced. The combinations by molar of PS-b-PAA and PS-b-PEO, 100:0, 75:25, 50:50, 25:75, and 0:100, were applied to establish the patched-like structure. Consequently, the films were examined the surface chemicals by chemical force spectroscopy mapping (CFSM) based on atomic force microscopy. The probe coated by the protein of poly-l-lysine to enhance the adhesive interaction between the probe and the surface was applied on the surface spatially and revealed the features of adhesive domains down to nanoscale. Area fraction of PAA decreases from 90% to 10% corresponding to the amount of PAA, as well as the PAA domain decreasing from 2 to 0.02 μm^2. Because PAA offers protein immobilization, meanwhile PEO prevents protein adsorption, therefore dBCP combinations show differences in concentration of protein lysates. Adherent cells like mesenchymal stem cell, in future work, will be cultured on the films and examined responses, like morphology, proliferation, and differentiation.

Emerging Single Molecule Techniques I

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Long Term Single Molecule TIRF Observation of Biomolecules without Immobilization

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Single-molecules fluorescence techniques have yielded valuable structural and dynamical information about molecular biological machines. The most common approaches used to observe single molecules in biology are confocal spectroscopy of freely diffusing molecules and total internal reflection (TIR) microscopy of surface attached molecules. With the latter technique it is possible to perform long-term observation of individual molecules, but at the cost of complex immobilization procedures that cannot be generally applied and often perturb the sample. Here we demonstrate a versatile technology that combines the advantages of both existing approaches, i.e. freely diffusing molecules and long-term observation with high signal to noise, in a simple, easy to handle, low-cost nanofluidic platform. Biomolecules flow through channels that are less than 100 nm deep, which keeps them within the TIR field, as required to fully exploit the high signal to noise possible with this optical sectioning technique. Rather than employing typical expensive nanofluidic technology, the nanochannels are generated using controlled channel collapse on a microfluidic device made from PDMS, ensuring easy fabrication and handling. We demonstrate that biomolecules labeled with single fluorophores can be imaged with millisecond time-resolution for several seconds. In addition, the design of the control channel of the device makes it possible to achieve high fluorophore photostability by removing oxygen without any need to add chemicals. By having a continuous stream of biomolecules flowing through the nanochannels, thousands of biomolecules can be measured each hour, illustrating the potential for automated experiments. We demonstrate the compatibility of the device with large biological complexes by showing multi-channel imaging of freely diffusing single nucleosomes.

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Sensing Cooperativity in ATP Hydrolysis for Single Multisubunit Enzymes in Solution

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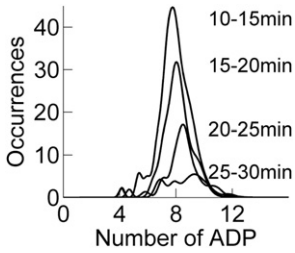
Cooperative interactions are critical for multisubunit enzymes to fulfill their enzymatic cycle in a coordinated fashion. To study this poorly understood process in the mammalian double-ring 16-subunit chaperonin TRiC/CCT, ATP number distributions in various hydrolyzed states are measured for single copies of the enzyme as each of the subunits can bind and hydrolyze ATP. Fluorescent-nucleotide-bound chaperonins are localized in free solution by closed-loop feedback provided by an Anti-Brownian ELectrokinetic trap (ABEL trap), producing fluorescence emission traces which allow determination of the number of nucleotides on each enzyme. As ADP molecules are dissociating from the chaperonin, the single peak at eight ADP bound simply falls in height over time, indicating a highly cooperative ADP release process difficult to observe by ensemble-averaged methods (figure).

By adding AlFx during ATP incubation, ATP transition state mimics (ADP•AlFx) are locked to the complex and show a dominant peak at 8 nucleotides for all incubation concentrations above 25 μM. Although ensemble averages of the single-molecule data can be matched with standard cooperativity models, surprisingly, the observed number distributions depart significantly from standard models and reveal stronger cooperativity, illustrating the power of the single-molecule distribution-based approach.



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Highly Parallel Magnetic Tweezers for the Study of DNA-Protein Interactions

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Single-molecule force-spectroscopy techniques, such as magnetic and optical tweezers, have become a powerful tool in the detailed investigation of the biophysical properties of DNA and its interaction with proteins. Typically, the truly single molecule nature of these experiments heavily limits data collection. However, the particle image based data analysis used in magnetic tweezers naturally lends itself to the simultaneous measurement of multiple, spatially separated, molecules.

We will describe the technical challenges involved in performing these multiplexed magnetic tweezers measurements and present the solutions. These include, the importance of post-experiment rather than real-time tracking due to computation constraints, the rapid selection of singularly tethered beads, and limits on the density of DNA-tethered magnetic beads. As a solution to the latter we describe a novel method to create targeted, non-random immobilization of DNA-tethered magnetic beads in regular, high density, arrays through micro-contact printing of DNA end-binding labels. This technique can allow an order of magnitude increase in data throughput of magnetic tweezers experiments. We are able to demonstrate the use of these techniques to perform experiments on hundreds of potential DNA-tethered beads in parallel resulting in statistical data on the mechanical properties of the DNA.

Having developed the technique we describe its application to investigating an essential process in the repair of DNA damage through homologous recombination. Specifically, we study the nucleation of RecA proteins onto DNA and the subsequent formation of nucleoprotein filaments. With nucleation occurring only once per molecule per experiment our multiplexed approach is ideally suited to provide insights into this process along with the potential to capture other statistically rare protein-DNA interactions.

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New Tools for Discovering the Role sRNA Plays in Cell Regulation

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Small RNA (sRNA, [1]) is a recently discovered class of small molecules recognized as an important regulator of cellular response. Direct study of sRNA dynamics within living cells faces two important challenges. First, the functional role of sRNA can be difficult to discern, as a given cell response or observable phenotype could have produced from a variety of possible regulatory network motifs. Second, the small size of sRNA makes it difficult to attach enough fluorescent probes to achieve a measurable signal without perturbing the system dynamics. To address these challenges, we have used single molecule fluorescence in situ hybridization (smFISH, [2]) to study cell-to-cell heterogeneity of mRNA copy numbers for human host cells in the presence and absence of bacterial sRNA. These experimental mRNA distributions are used to refine and down-select regulatory model that are evaluated by the Finite State Approach [3] along with other theoretical techniques. A large number a cells are subjected to varying conditions such as nutrient concentration, salt content, and pathogen infection